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(54) Photoanalysis apparatus with means for correcting flow rate of fluid carrying particles and/or position of applied light beam for examination and photoanalysis method therefor.

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Description

The present invention relates to a sheath flow type photoanalysis apparatus in which fluid carrying particles such a cells flow in laminar conditions surrounded by sheath fluid through a capillary tube, a light beam having a constant wave length is applied on the flow of the fluid carrying particles, and properties of the particles such as kind, size, number and shape are measured form strength of light scattering and/or fluorescence caused by the particles and, more particularly, to a sheath flow type photoanalysis apparatus with means for correcting a position of the applied light beam for examination and the flow rate of the fluid carrying particles.

The present invention also relates to a method for corrcecting a position on which a light beam for examination is applied and for correcting flow rate of fluid carrying particles in photoanalysis apparatus.

For example, US-patent No. 3,873,204 SCIENCE (Vol. 150, pp. 630-631, October 1965), and Rev. Sci. Instrum. (Vol. 46, pp. 1021-1024, No. 8, August 1975) have disclosed the photoanalysis apparatus in which fluid carrying particles such as cells flow through a capillary tube in laminar conditions surrounded by the sheath fluid, a light beam having a constant wave length is applied on the flow of the fluid carrying particles, and properties of the particles such as kind, size, number and shape are measured from strength of light scattering and/or fluorescence caused by the particles.

In the above-mentioned prior arts, flow of fluid carrying pilot particles such as control cells (for example, spherical cells such as blood of a chicken) or reference particles (spherical artificial particles) are subjected to optical measurement before the subject examination of particles starts and, from the result of the measurement an appropriate position on which the light beam for examination is applied is experientially determined. That is to say, since the flow rate of the fluid carrying particles to be examined is preset by utilizing the pilot particles which are different from the actual particles to be examined, the conventional method lacks reliability. Further, the operations for deciding the position on which the light beam is applied become thus tedious and complicated. When a difference in position occurs between the position on which the light beam for examination is applied and the flow of the fluid carrying particles by any disturbance during the actual examination of the particles, it is almost impossible to correct such a difference in position with the examination being continued.

In order to conduct the optimum optical measurement, it is necessary that the particles carried in the fluid flow through a spot of the light beam for examination. Thus, a width of the flow of the fluid carrying particles should appropriately be maintained constant. The width of the fluid carrying particles depends on its flow rate. Accordingly, there exists an optimum value to the flow rate of the fluid carrying particles in order to conduct an optimum optical measurement. Whereas, in the prior arts, the pilot particles are streamed prior to the examination of the particles to be examined and, the optimum value of the flow rate is supposed to be determined from the results of the optical measurement of the strength of light scattering and/or fluorescence caused by the pilot particles. Since the flow rate of the fluid carrying particles is determined by the pilot particles different from the actual particles to be examined, the prior art method has poor reliability. Further, the prior art method has a disadvantage that when a variation of the flow rate is occurred by any cause during the examination, it is not possible to correct the variation of the flow rate during the examination.

EP-A-0210343 discloses a flow cytometry apparatus for determining characteristics of particles flowing in a stream. A light source provides a beam of focused light to illuminate the particles moving in the stream. A beam steering member is positioned to adjust the focal point of the light beam on the particles by refracting the light beam to cause a displacement of the focal point. The beam steering member cannot be adjusted automatically. The flow rate of the stream cannot be controlled.

The object of the present invention is to overcome the disadvantages of the prior art and to provide a sheath flow type photoanalysis apparatus with improved control means.

The present invention achieves this object with an apparatus according to the first claim and a method for correcting according to claim 4.

A sheath flow type photoanalysis apparatus according to the present invention is characterized in that it comprises marker pouring means for pouring markers into one of sheath fluid and fluid carrying particles in a flow-cell device, the markers emitting fluorescence or scattered light when said markers receive a light beam for correction; optical means for applying the light beam for correction to the markers in the flow-cell device to measure strength of fluorescence or scattered light caused by the markers to generate a signal for correction; signal processing means for calculating from the signal for correction at least one of a difference in position between a center of the fluid carrying particles in the flow-cell device and a center of the light beam for examination and a difference in flow rate between a predetermined flow rate and an actual flow rate of the fluid carrying particles to generat correspondingly at least on of a command signal for position correction and a command

signal for flow rate correction; and correspondingly at least one of first correcting means for shifting the center of the light beam for examination to the center of the fluid carrying particles in accordance with the command signal for position correction and second correcting means for correcting the flow rate of the fluid carrying particles in the flow-cell device to the predetermined flow rate in accordance with the command signal for flow rate correction.

In one embodiment of the present invention, the marker pouring means includes an opening opened in the flow of the fluid carrying particles; the optical means comprises a scanner with a scanner mirror which is disposed between a lamp and a condenser lens for oscillating the light beam for examination in crosswise direction of the flow of the fluid carrying particles within a predetermined angle, a first flat half mirror disposed between the scanner mirror and the condenser lens, the first flat half mirror permitting the light beam for examination to pass therethrough and reflecting a light beam from opposite direction of the light beam for examination, a curved half mirror disposed between the first flat half mirror and the condenser lens, whose concave surface is faced toward the condenser lens, the curved half mirror permitting a part of the light beam for examination to pass therethrough and reflecting a remaining part of the light beam for examination, a second flat half mirror disposed between an objective lens and a first photo-detector, which reflects only fluorescence or light scattering caused by the markers in the fluid carrying particles, and a second photo-detector for detecting the fluorescence or light scattering reflected by the second flat half mirror to generate a signal for correction; the first correcting means comprising a scanner controller to control the scanner to change a center of the oscillation in accordance with said first command signal; and the second correcting means comprising a valve controller to control a flow control valve to correct the flow rate of the fluid carrying particles in the flow-cell device in accordance with the second command signal.

In another embodiment of the present invention, the marker pouring means includes two openings respectively opened at each side of the flow of the fluid carrying particles in the flow-cell device to pour from these two openings different markers which emit fluorescence or light scattering whose wave lengths are different from each other; the second optical means comprises a mirror disposed between the lamp and the condenser lens, the mirror being rotatable so as to move the light beam for examination in crosswise direction of the flow of the fluid carrying particles, a diffraction grating disposed between the mirror and the flow-cell device for separating two light beams for correction from the light beam for examination, the two light beams for correction respectively being applied on the markers, a flat half mirror disposed between the objective lens and the first photo-detector, the flat half mirror reflecting only fluorescence or scattered light caused by the markers in the sheath fluid, and a second photo-detector for detecting the fluorescence or scattered light reflected by the flat half mirror to generate the signal for correction; the first correction means comprises a controller for moving the mirror in accordance with the first command signal; and the second correcting means comprises a valve controller to control the flow control valve to correct the flow rate of the fluid carrying particles in the flow-cell device in accordance with the second command signal.

A method for correcting a difference in position between a center of flow of fluid carrying particles and a center of a light beam for examination in a sheath flow type photoanalysis apparatus according to the invention comprises: pouring markers which emit fluorescence or scattered light having a constant wave length when said markers receive a light beam; applying the light beam for examination of the flow of the fluid carrying particles; detecting strength of the fluorescence or scattered light caused by the markers in the fluid carrying particles to generate a signal for correction; calculating a difference in position between a center of the fluid carrying particles in the flow-cell means and a center of said light beam for examination and/or a difference in flow rate between a predetermined flow rate and an actual flow rate of said fluid carrying particles in said flow-cell means to generate correspondingly a first command signal for position correction and/or a second command signal for flow rate correction; and correspondingly shifting the center of the light beam for examination to the center of said fluid carrying particles in said flow-cell means in accordance with said first command signal and/or correcting the flow rate of said fluid carrying particles to the predetermined flow rate in accordance with said second command signal.

A method for correcting flow rate of flow of fluid carrying particles in a sheath flow type photoanalysis apparatus according to the invention comprises: pouring makers, which emit fluorescence or scattered light having a constant wave length when said markers receive a light beam, into the fluid carrying particles; oscillating the light beam for examination in crosswise direction of the flow of the fluid carrying particles within a predetermined angle (α).

Another method for correcting flow rate of flow of fluid carrying particles in a sheath flow type photoanalysis apparatus according to the invention

comprises: pouring different markers, which emit fluorescence or scattered light having constant wave lengths different from each other when said markers receive a light beam, into a sheath fluid at each side of the flow of the fluid carrying particles, separating two light beams for correction from the light beam for examination by means of a diffraction grating to respectively apply these two light beams on the markers at each side of the fluid carrying particles in such a manner that centers of the two light beams for correction are offset from centers of the flows of the markers opposite to each other. of the fluorescence or light scattering caused by the markers in the sheath fluid to generate a signal for correction; calculating a difference in position between a center of the fluid carrying particles in the flow-cell means and a center of the light beam for examination on the basis of the signal for correction to generate a command signal for position correction; and shifting the center of the light beam for examination to the center of the fluid carrying particles in accordance with the command signal.

A method for correcting flow rate of flow of fluid carrying particles in a sheath flow type photoanalysis apparatus according to the invention comprises: pouring markers, which emit fluorescence or light scattering having a constant wave length when received a light beam, into the fluid carrying particles; oscillating the light beam for examination in crosswise direction of the flow of the fluid carrying particles; detecting strength of the fluorescence or light scattering caused by the marker in the fluid carrying particles to generate a signal for correction; calculating a difference in the flow rate between a predetermined flow rate and an actual flow rate of the fluid carrying particles on the basis of the signal for correction to generate a command signal for flow rate correction; and correcting the flow rate of the fluid carrying particles to the predetermined flow rate in accordance with the command signal.

Another method for correcting flow rate of flow of fluid carrying particles in a sheath flow type photoanalysis apparatus according to the invention comprises: pouring different markers, which emit fluorescence or light scattering having constant wave lengths different from each other when received a light beam, into a sheath fluid at each side of the flow of the fluid carrying particles; separating two light beams for correction from the light beam for examination by means of a diffraction grating to respectively apply these two light beams on the markers at each side of the fluid carrying particles in such a manner that centers of the two light beams for correction are offset from centers of the flows of the markers opposite to each other; detecting strength of the fluorescence or light scattering caused by the markers in the sheath fluid to generate a signal for correction; calculating a difference in flow rate between a predetermined flow rate and an actual flow rate of the fluid carrying particles on the basis of the signal for correction to generate a command signal for flow rate correction; and correcting the flow rate of the fluid carrying particles to the predetermined flow rate in accordance with the command signal.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematically illustrated explanatory view showing one embodiment of a sheath flow type photoanalysis apparatus according to the present invention;

Fig. 2 is a schematically illustrated plan view of a flow-cell device incorporated in the embodiment shown in Fig. 1;

Fig. 3 is an enlarged view of an essential portion of the embodiment shown in Fig. 1, illustrating the operations of the photoanalysis apparatus, in which a condenser lens, the main body of the flow-cell device, a scanner and some others are conveniently omitted for clarification;

Figs. 4-1-a~e, Figs. 4-2-a~e, Figs. 4-3-a~e, and Fig. 4-4 are illustrative views and graphs, which explain a method for correcting a difference in position between a center of flow of fluid carrying particles and a center of a light beam for examination in the apparatus of one embodiment of the present invention, respectively;

Figs. 5-1-a~e, Figs. 5-2-a~e, Figs. 5-3-a~e, and Fig. 5-4 are illustrative views and graphs, which explain a method for correcting flow rate of the fluid carrying particles in the apparatus of one embodiment of the present invention, respectively;

Fig. 6 is a schematically illustrated explanatory view showing another embodiment of the sheath flow type photoanalysis apparatus according to the present invention;

Fig. 7 is a schematically illustrated plan view of a flow-cell device used for the embodiment in Fig. 6; and

Fig. 8a and Fig. 8b are enlarged views of a portion A in Fig. 7, each showing an applying mode of the light beam for correction to the flow of markers in the sheath fluid.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One embodiment of the sheath flow type photoanalysis apparatus according to the present invention will be described hereinafter with reference to Figs. 1 - 5.

A flow-cell device 6 includes two inlet openings

6a, 6b for sheath fluid 23, an inlet opening 6c for fluid 22 carrying particles formed between these two inlet openings and a fluid outlet opening 6d and the fluid 22 carrying particles is surrounded by the sheath fluid 23 and is made to flow through the flow-cell device 6 in laminar conditions. Connected to the inlet opening 6c for the fluid 22 carrying particles are a flow regulating valve 13 which controls a flow rate of the fluid 22 carrying particles and marker pouring device 10 which pours markers into the fluid 22 carrying particles. The markers are of fluorescence materials which emit fluorescence having a constant wave length different from those of fluorescence emitted by the particles in the fluid carrying particles when received a light beam. Upper and lower surfaces of the flow-cell device 6 are transparent so that the light beam can pass through. On one side of the flow-cell device 6, there is provided a lamp 1 emitting the light beam 18 for examination. A scanner mirror 2 is connected to a scanner 15 and structured so as to oscillate the light beam 18 for examination projected from the lamp 1 at high frequency in crosswise direction of the flow of the fluid 22 carrying particles in the flow-cell device 6. A condenser lens 5 is disposed on an optical axis of the light beam 18 for examination between the scanner mirror 2 and the flow-cell device 6 so as to condense the light beam 18 for examination to the fluid 22 carrying particles which flows through the flow-cell device 6 in laminar conditions. A first flat half mirror 3 is disposed between the scanner mirror 2 and the condenser lens 5 and is structured to permit the light beam for the examination supplied from the side of the scanner mirror 2 to pass therethrough and, on the contrary, to reflect a light beam from the opposite direction. A curved half mirror 4 is interposed between the first flat half mirror 3 and the flow-cell device 6 in such a manner that its concave surface is faced toward the flow-cell device 6, and a center line of the curve extends parallel to the flow of the fluid 22 carrying particles in the flow-cell device 6. This curved half mirror 4 is such arranged that a part of the light beam from the side of the first flat half mirror 3 is reflected therefrom and a remaining part of the light beam passes therethrough.

An objective lens 7 is provided on the side opposite to the condenser lens 5 of the flow-cell device 6 in order to collect a light beam 19 containing fluorescence 20 from the markers poured into the fluid 22 carrying particles and light scattering and/or fluorescence 21 from the particles 24 in the fluid 22 carrying particles. A second flat half mirror 8 is provided on the downstream side of the objectiv lens 7. The second flat half mirror 8 allows the light scattering and/or the fluorescence 21 caused by the particles 24 in the fluid 22 carrying particles in a light beam 19 collected by the objective lens 7 to pass therethrough. That is to say, the collected light beam 19 is separated into the light scattering and/or the fluorescence 21 for examination of the particles and the fluorescence 20 for correction. A first photo-detector 16 is located at the downstream side of the second flat half mirror 8 and detects the strength of the light scattering and/or the fluorescence 21 for examination passed through the second flat half mirror 8 to convert it into the signal for examination. A first signal processor 17 is connected to the first photo-detector 16 to analysis with the signal from the first photo-detector 16 some properties of the particles 24 in the fluid 22 carrying particles.

A second photo-detector 9 is placed adjacent to the second flat half mirror 8 and detects the strength of the fluorescence 20 for correction separated by the second flat half mirror 8 to generate a signal for correction. A second signal processor 11 is connected to the second photo-detector 9 and, on the basis of the signal for correction from the second photo-detector 9, it calculates a difference in position between a center of the fluid 22 carrying particles in the flow-cell device 6 and a center of the oscillation of the light beam 18 for examination to generate a command signal for correction and also a difference in flow rate between the predetermined flow rate and an actual flow rate of the fluid 22 carrying particles in the flow-cell device to generate a command signal for flow rate correction. Connected to the second signal processor 11 are a valve controller 12 for controlling a flow regulating valve 13 and a scanner controller 14 for controlling the oscillation of the scanner 15. The valve controller 12 controls in accordance with the command signal for flow rate correction the flow regulating valve 13 to correct the flow rate of the fluid 22 carrying particles in the flow-cell device 6 and the scanner controller 14 controls in accordance with the command signal for position correction to correct a center of the oscillation of the light beam 18 for examination toward a center of the flow of the fluid 22 carrying particles in the flow-cell device.

The light beam 18 for examination emitted from the lamp 1 is oscillated to the extent not causing variation of the light scattering and/or fluorescence for examination. In other words, the light beam 18 for examination is oscillated only within a very small angle α to the extent that the particles 24 in the carrying fluid 22 may not deviate out of the spot of the light beam 18 for examination. The light beam 18 for examination passes through the first flat half mirror 3 and the curved half mirror 4 and is applied to the fluid 22 carrying particles in the flow-cell device 6. At this time, parts 25 of the light beam 18 for examination are reflected by the curved half mirror 4 and then are again reflected

by the first flat half mirror 3 to pass through the curved half mirror 4 with being attenuated at a certain extent and finally are applied on locations remote from the flow of the fluid 22 carrying particles in crosswise direction. That is to say, the light beam for correction is an oscillated light beam for examination. As the second flat half mirror 8 permits only the light scattering and/or the fluorescence 21 caused by the particles 24 in the fluid 22 to pass therethrough, the fluorescence 20 caused by the markers is transmitted to the second photo-detector 9. Then the transmitted fluorescence 20 is converted into a signal for correction as amplitude modulation of a carrier wave caused by an oscillator included within the second photo-detector 9. The signal for correction is input in the second signal processor 11, and delivered to one processing system which calculates a difference in position between the center of the flow of the fluid 22 carrying particles and the center of the oscillation of the light beam 18 for examination to generate a command signal for position correction, and to the other processing system which calculates a difference in flow rate between the predetermined flow rate and the actual flow rate of the fluid 22 carrying particles to generate a command signal for flow rate correction.

The correcting steps in the processing system for position correction will be explained with reference to Figs. 4-1-a~e, Figs. 4-2-a~e, Figs. 4-3-a~e, and Figs. 4-4. When the reflected light beam 25 is oscillated between a point A and a point B, as shown in Fig. 4-1-a,, it is assumed that the fluid 22 carrying particles is shifted to the side of A, as shown in the same drawing. At the moment, a peak value V of amplitude of carrier wave of the signal for correction varies between both A and B points as shown in Fig. 4-1-b. When the abscissa is set as the axis of time t, the peak to peak value V turns into Fig. 4-1-c. When this peak to peak value V is multiplied by an oscillation amplitude W (Fig. 4-4) of the scanner 15 through an analog multiplier, an output M as shown in Fig. 4-1-d is obtainable. When a direct current component is taken out from the output M while passing through a high-frequency cut filter, a negative value is obtained as shown in Fig. 4-1-e.

When the same procedures as in Figs. 4-1-a~e are applied to a case in which the fluid 22 carrying particles flows at a center of the A-B points (Figs. 4-2-a~e) and a case in which the fluid 22 carrying particles flows at a side of B point between the A-B points, respective outputs $\underline{M} = 0$ and $\underline{M} > 0$ can be obtained.

When th output $\underline{M}$ is used to impress as a voltage controlling a center of amplitude of the scanner controller 14 through an amplifier, it becomes possible to register the center of the amplitude between the points A and B automatically with the center of the flow of the fluid 22 carrying particles. That is to say, the light beam 18 for examination is continuously applied to the center of the flow of the fluid 22 carrying particles without using the pilot particles.

The correcting steps in the processing system for the flow rate correction will be explained with reference to Figs. 5-1-a~f, Figs. 5-2-a~f, Figs. 5-3-a~f, and Fig. 5-4. When the reflected light beam 25 is oscillated between a point A and a point B, as shown in Fig. 5-1-a, it is assumed that the fluid 22 carrying particles is increased in flow rate and is enlarged in width, as shown in the same drawing. At the moment, a peak to peak value V of the carrier wave of the signal for correction varies between both A and B points such as shown in Fig. 5-1-b. When the abscissa is set as the axis of time t, the peak to peak value V turns into a signal shown in Fig. 5-1-c. When a direct current component is taken out from this signal and a negative value is reversed into a positive value, that is, a variation component of the signal is rectified, a signal V' (Fig. 5-1-d) is obtained. When this signal V' is multiplied by an amplitude W' (Fig. 5-4) of a wave which has a frequency twice the oscillation frequency of the scanner 15 and advanced at $\pi/2$ through the analog multiplier, an output M' as shown in Fig. 5-1-e is obtained. When a direct current component is taken out from the output M' by means of a high-frequency cut filter, a positive output $\underline{M'}$ is obtained as shown in Fig. 5-1-f.

The same procedures as in Figs. 5-1-b-f are applied to a case in which the flow rate of the fluid 22 carrying particles substantially equals to a predetermined value (Figs. 5-2-a~f) and a case in which the flow rate is less than such a predetermined value (Figs. 5-3-a~f), respective outputs $\underline{M'} = 0$ and $\underline{M'} < 0$ can be obtained.

When this output $\underline{M'}$ is used to impress as a voltage driving the valve controller 12 through the amplifier, the flow rate of the fluid 22 carrying particles can be automatically maintained at the predetermined flow rate. Further, by changing the oscillation amplitude between the A-B points, a predetermined flow rate of the fluid 22 carrying particles can be freely selected.

When the oscillation frequency of the scanner mirror 2 is set at a high frequency such as 1 KHz, it is possible to shift the center of the light beam for examination 18 to the center of the flow of the fluid 22 carrying particles with a high responsibility even if th flow of the fluid carrying particles is shifted by any turburance to the flow-cell device 6, clogging of the flow system and the like.

The control operations such as adjustment of the optical axes before the examination may be almost eliminated.

Next, another embodiment of the sheath flow type photoanalysis apparatus according to the present invention will be described hereinafter with reference to Figs. 6 - 8. Explanations to the same portions coincident with those in the embodiment of Fig. 1 will be omitted.

The flow-cell device 6 includes two inlet openings 6a, 6b for the sheath fluid 23, an inlet opening 6c for the fluid 22 carrying particles formed between these two inlet openings, two inlet openings 28a, 28b for the markers each provided in a flow passage for the sheath fluid and the fluid outlet opening 6d. A flow regulating valve 13 which controls flow rate of the fluid carrying particles is connected to the inlet opening 6c for the fluid carrying particles. Further, marker pouring devices 10a, 10b which pour the markers into the sheath fluid 23 are connected to the inlet openings 28a, 28b for the markers, so that flows 31, 32 of the markers are provided at both sides of the fluid 22 carrying particles. The markers poured through the marker pouring device 10a, 10b are different markers which emit fluorescence having constant wave lengths different from each other when received a high beam. The reason why the wave lengths are made to be different from each other is to make it possible to detect a direction of difference in position between the fluid 22 carrying particles and the light beam 18 for examination.

Although, in the above-mentioned embodiment there are provided the first flat half mirror 3 and the curved half mirror 4 between the scanner mirror 2 and the condenser lens 5, this embodiment includes a diffraction grating 26 by which two light beams 29, 30 for correction are separated from the light beam 18 for examination and applied to the sheath fluid 23 in the flow-cell device 6. The light beams 29, 30 for correction are applied in such a manner that centers of the light beams 29, 30 for correction are offset from centers of flow of the markers 31, 32 in a direction opposite to each other as shown in Figs. 8a and 8b. A controller 27 is connected to the scanner mirror 2 to rotatably move the scanner mirror 2 in accordance with the command signal for position correction from the signal processor 11. That is to say, an optical axis of the light beam 18 for examination is movable by the rotational movement of the scanner mirror 2 in a direction perpendicular to the flow of the fluid 22 carrying particles in the flow-cell device 6.

The correcting process will be explained by taking a case as an example in which a manner of the light beams for correction applied is in the manner shown in Fig. 8a.

When a position of the flow of the fluid 22 carrying particles is shifted rightwardly of the drawing, the flow 31 of the markers goes out of the spot of the light beam 29 for correction, whereas the flow 32 of the markers comes into the spot of the light beam 30 for correction. As a result, the fluorescence from the flow 31 of the markers is decreased in strength, while the fluorescence from the flow 32 of the markers is increased in strength. When a position of the flow of the fluid 22 carrying particles is shifted leftwardly of Fig. 8a, the flow 31 of the markers comes into the spot of the light beam 29 for correction, whereas the flow 32 of the markers goes out of the spot of the light beam 30 for correction. As a result, the fluorescence from the flow 31 of the markers is increased in strength, while the fluorescence of the markers is decreased in strength. Since, as mentioned above, there exists the difference in wave length between the fluorescence from one markers 31 and the fluorescence from the other markers 32, each fluorescence can thus be distinguished mutually. Thus when the variation in strength of the fluorescence is measured, an offset direction and a difference in position between the center of the light beam 18 for examination and the center of the flow of the fluid 22 carrying particles can be detected. These fluorescence is separated by the half mirror 8 from the light beam 19 which is collected through the objective lens 7 and, the separated fluorescence is transmitted to the second photo-detector 9 and converted into a signal for correction. The signal processor 11 connected to the second photo-detector 9 calculates from the signal for correction the offset direction and the difference in position between the center of the flow of the fluid 22 carrying particles in the flow-cell device 6 and the center of the light beam 18 for examination and, then, it outputs a command signal for position correction to the controller 27. The controller 27 rotates the scanner mirror 2 in accordance with the command signal for position correction to shift the center of the light beam 18 for examination toward the center of the flow of the fluid 22 carrying particles 18.

When the flow rate of the fluid 22 carrying particles increases, both flows 31 and 32 of the markers are moved away from each other so that the flows 31, 32 of the markers go out of the respective spots of the light beams for correction 29, 30. The fluorescence from the both markers are thus weakened in strength at the same time. Furthermore, when the flow rate of the fluid 22 carrying particles decreases, the flows 31 and 32 of the markers approach to each other so that both flows 31, 32 come into the spots of the light beams 29, 30 for correction. Each strength of the fluorescence from both markers thus increases simultaneously.

With the same manner as described previously, these fluorescence is separated by the half mirror 8 from the light beam 19 which is collected through the objective lens 7 and, the separated fluoresc nc is transmitted to the second photo-

detector 9 and converted into a signal for correction. The signal processor 11 connected to th second photo-detector 9 calculates from the signal for correction the difference in flow rate between the actual flow rate of the fluid 22 carrying particles in the flow-cell device 6 and the predetermined flow rate and, then, it outputs a command signal for flow rate correction to the valve controller 12. The valve controller 12 controls the flow regulating valve 13 in accordance with the command signal for flow rate correction to regulate the flow rate of the fluid 22 carrying particles in the flow-cell device 6 to the predetermined flow rate.

In this embodiment, the applying position of the light beam for examination and the flow rate of the fluid carrying particles can be set without using the pilot particles.

In the above embodiments, the fluorescent materials emitting fluorescence having constant wave lengths when received the light beam have been employed as markers which represent the position and flow rate of the fluid 22 carrying particles, but the reference particles, control cells and the like may, of course, be utilized with such materials.

Also, in the above-mentioned embodiments, although both of the means for position correction and flow rate correction have been accompanied with the apparatus, the apparatus may incorporate either one of these means.

As fully described in hereinbefore, according to the present invention, the applying position of the light beam for examination as well as the flow rate of the fluid carrying particles are determined by means of the subject particles to be examined, thereby substantially eliminating the tedious adjustment operations such as adjustments of the light applying position and flow rate using the pilot particles before the examination.

Further, according to the invention, it is possible to shift the center of the light beam for examination 18 to the center of the flow of the fluid 22 carrying particles with a high responsibility even if the flow of the fluid carrying particles is shifted by any turburance to the flow-cell device 6 of clogging of the flow system and the like.

Further, according to the invention, it is possible to always maintain the flow rate of the fluid 22 carrying particles at a predetermined flow rate at a position on which the light beam 18 for examination is applied even if causes causing the variation of the flow rate such as clogging of the flow system, reduction of pressure to supply the fluid carrying particles are occurred during the examination.

Claims

1. A photoanalysis apparatus comprising:
flow-cell means for pouring sheath fluid (23) and fluid (22) carrying particles (24) in laminar flow in which said fluid (22) carrying particles is surrounded by said sheath fluid (23), said flow-cell means including a flow-cell device (6), and
first optical means (1, 5, 7, 16) for applying a light beam (18) for examination having a constant wave length on said fluid (22) carrying particles in the flow-cell device (6) to measure strength of light scattering or fluorescence caused by said particles (24) in said fluid (22) carrying particles to generate a signal for examination, said first optical means comprising a lamp (1) for emitting said light beam (18) for examination, a condenser lens (5) disposed between said flow-cell device (6) and said lamp (1), an objective lens (7) disposed on opposite side of said flow-cell device (6), and a first photo-detector (16) to measure the strength of light scattering or fluorescence caused by the particles (24) in the fluid (22) carrying particles to generate the signal for examination; and
first signal processing means (17) for processing said signal for examination from said first optical means to detect properties of the particles such as kind, size, number and shape;
characterized by
marker pouring means (10; 10a, 10b) for pouring markers into said sheath fluid (23) and fluid (22) carrying particles in said flow-cell device (6), said markers emitting fluorescence and/or scattered light having a constant wave length when said markers receive a light beam (18; 29, 30) for correction;
a flow control valve (13) to control the flow rate of said fluid (22) carrying particles;
second optical means (2, 8, 9, 15; 26, 27) for applying the light beam for correction to said markers in said flow-cell device (6) to measure strength of fluorescence and/or scattered light caused by said markers to generate a signal for correction;
second signal processing means (11) for calculating from said signal for correction a difference in position between a center of said fluid carrying particles in said flow cell device and a center of said light beam for examination and/or a difference in flow rate between a predetermined flow rate and an actual flow rate of said fluid carrying particles in said flow-cell device to generate correspondingly a first command signal for position correction and/or a second command signal for flow rate correction; and
correspondingly first correction means (14) for shifting the center of the light beam for examination to the center of said fluid carrying par-

ticles in said flow-cell device in accordance with said first command signal and/or second correcting means (12) for correcting the flow rate of said fluid carrying particles to the predetermined flow rate in accordance with said second command signal.

2. A photoanalysis apparatus as claimed in claim 1, wherein said marker pouring means (10) includes an opening opened in the flow of said fluid carrying particles, and
said second optical means comprises a scanner (15) with a scanner mirror (2) which is disposed between said lamp and said condenser lens for oscillating said light beam for examination in crosswise direction of the flow of the fluid carrying particles within a predetermined angle (α), said light beam for correction being identical with said oscillated light beam for examination, a first flat half mirror (3) disposed between said scanner mirror and said condenser lens which permits said light beam for examination to pass therethrough and reflects a light beam from opposite direction of said light beam for examination, a curved half mirror (4) disposed between said first flat half mirror and said condenser lens of which concave surface is faced toward said condenser lens, said curved half mirror permitting a part of said light beam for examination to pass there-through and reflects a part of said light beam for examination, a second flat half mirror (8) disposed between said objective lens and said first photo-detector which reflects only fluorescence or scattered light caused by said markers in said fluid carrying particles, and a second photo-detector (9) for detecting said fluorescence or light scattering reflected by said second flat half mirror to generate said signal for correction, and
said first correcting means comprises a scanner controller (14) to control said scanner to change a center of said oscillation in accordance with said first command signal, and
said second correcting means comprises a valve controller (12) to control said flow control valve to correct the flow rate of said fluid carrying particles in said flow-cell device in accordance with said second command signal.

3. A photoanalysis apparatus as claimed in claim 1, wherein
said marker pouring means includes two openings (28a, 28b) respectively opened at each side of the flow of the fluid carrying particles in said flow-cell device and pours from said two openings into the sheath fluid different markers which emit fluorescence or scattered light of which wave lengths are different form each other, and
said second optical means comprises a mirror (2) disposed between said lamp and said condenser lens, said mirror being rotatable so as to move said light beam for examination in crosswise direction of the flow of the fluid carrying particles, a diffraction grating (26) disposed between said mirror and said flow-cell device for separating two light beams (29, 30) for correction from said light beam for examination, said two light beams for correction respectively being applied on flow (31, 32) of the markers in such a manner that respective centers of the two light beams for correction are offset form respective enters of the flow of the markers in a direction opposite to each other, a flat half mirror (8) disposed between said objective lens and said first Photo-detector, said flat half mirror reflecting only fluorescence or scattered light caused by said markers in said sheath fluid, and, a second photo-detector (9) for detecting fluorescence or scattered light reflected by said flat half mirror to generate said signal for correction, and
said first correcting means comprises a controller (27) for moving said mirror in accordance with said first command signal, and
said second correcting means comprises a valve controller (12) to control said flow control valve to correct the flow rate of said fluid carrying particles in said flow-cell device in accordance with said second command signal.

4. A method for correcting a difference in position between a center of flow of fluid (22) carrying particles (24) and a center of light beam (18) for examination and/or for correcting a flow rate of the fluid (22) in a photoanalysis apparatus which comprises flow-cell means (6) for pouring sheath fluid (23) and the fluid carrying particles in laminar flow in which the fluid carrying particles is surrounded by the sheath fluid, optical means (1, 5, 7, 16) for applying the light beam for examination having a constant wave length on the fluid carrying particles in the flow-cell means to measure strength of light scattering and/or fluorescence caused by the particles in the fluid carrying particles to generate a signal for examination, and signal processing means (17) for processing the signal for examination from the optical means to detect properties of the particles such as kind, size, number and shape,
characterized by
pouring markers which emit fluorescence and/or scattered light having a constant wave length when the markers receiv a light beam

(18; 29, 30) into said sheath fluid (23) or fluid (22) carrying particles,
applying the light beam (18; 29, 30) to said markers in said flowcell means to measure strength of fluorescence and/or light scattering caused by said markers to generate a signal for correction;
calculating from said signal for correction a difference in position between a center of said fluid carrying particles in said flow-cell means and a center of said light beam for examination and/or a difference in flow rate between a predetermined flow rate and an actual flow rate of said fluid carrying particles in said flow-cell means to generate correspondingly a first command signal for position correction and/or a second command signal for flow rate correction; and
correspondingly shifting the center of the light beam for examination to the center of said fluid carrying particles in said flow-cell means in accordance with said first command signal and/or correcting the flow rate of said fluid carrying particles to the predetermined flow rate in accordance with said second command signal.

5. A correcting method according to claim 4, wherein
said marker pouring comprises pouring markers into said fluid (22) carrying particles and
said applying the light beam to said markers in said flow-cell means comprises oscillating said light beam (18) for examination in crosswise direction of the flow of the fluid carrying particles within a predetermined angle (α).

6. A correcting method according to claim 4, wherein
said marker pouring comprises pouring different markers which emit fluorescence and/or light scattering having constant wave lengths different from each other when received a light beam into the sheath fluid (23) at each side of the flow of the fluid (22) carrying particles, and
said applying the light beam to said markers in said flow-cell means comprises separating two light beams (29, 30) for correction from said light beam (18) for examination by means of a diffraction grating (26) to respectively apply onto said markers at each side of the fluid (22) carrying particles in such a manner that centers of the two light beams (29, 30) for correction are offset form centers of the flows (31, 32) of the markers in a direction opposite to each other.

Patentansprüche

1. Fotoanalyseapparatur mit
einer Strömungszelleneinrichtung zum Eingießen von Hüllenfluid (23) und Partikel-(24)-tragendem Fluid (22) in laminarer Strömung, in der das Partikel-tragende Fluid (22) durch das Hüllenfluid (23) umgeben ist, wobei die Strömungszelleneinrichtung eine Strömungszellenvorrichtung (6) beinhaltet, und
einer ersten optischen Einrichtung (1, 5, 7, 16) zum Anlegen eines Lichtstrahls (18) mit einer konstanten Wellenlänge zur Untersuchung an das Partikel-tragende Fluid (22) in der Strömungszellenvorrichtung (6), um die Stärke der durch die Partikel (24) in dem Partikel-tragenden Fluid (22) verursachte Stärke der Lichtstreuung oder Fluoreszenz zu messen, um ein Signal zur Untersuchung zu erzeugen, wobei die erste optische Einrichtung aufweist:
eine Lampe (1) zum Emittieren des Lichtstrahls (18) zur Untersuchung, eine zwischen der Strömungszellenvorrichtung (6) und der Lampe (1) angeordnete Kondensorlinse (5), eine an der gegenüberliegenden Seite der Strömungszellenvorrichtung (6) angeordnete Objektivlinse (7), und einen ersten Fotodetektor (16) zum Messen der Stärke der durch die Partikel (24) in dem Partikel-tragenden Fluid (22) verursachten Lichtstreuung oder Fluoreszenz, um das Signal zur Untersuchung zu erzeugen; und
einer ersten Signalverarbeitungseinrichtung (17) zum Verarbeiten des Signals zur Untersuchung von der ersten optischen Einrichtung, um Eigenschaften der Partikel, wie zum Beispiel Art, Größe, Anzahl und Form, zu erfassen;
gekenzeichnet durch,
eine Markierer-Eingießeinrichtung (10; 10a, 10b) zum Eingießen von Markierern in das Hüllenfluid (23) und Partikel-tragende Fluid (22) in der Strömungszellenvorrichtung (6), wobei die Markierer Fluoreszenz- und/oder Streulicht mit einer konstanten Wellenlänge emittieren, wenn die Markierer einen Lichtstrahl (18; 29, 30) zur Korrektur empfangen;
ein Strömungs-Steuerungsventil (13) zum Steuern der Strömungsrate des Partikel-tragenden Fluids (22);
eine zweite optische Einrichtung (2, 8, 9, 15; 26, 27) zum Anlegen des Lichtstrahls zur Korrektur an die Markierer in der Strömungszellenvorrichtung (6), um die Stärke der durch die Markierer verursachten Fluoreszenz und/oder des Streulichtes zu messen, um ein Signal zur Korrektur zu erzeugen;
eine zweite Signalverarbeitungs-Einrichtung (11), um von dem Signal zur Korrektur eine Positionsdifferenz zu berechnen zwischen ei-

nem Zentrum des Partikel-tragenden Fluids in der Strömungszellenvorrichtung und einem Zentrum des Lichtstrahls zur Untersuchung und/oder eine Differenz der Strömungsrate zwischen einer vorbestimmten Strömungsrate und einer tatsächlichen Strömungsrate des Partikel-tragenden Fluids in der Strömungszellenvorrichtung, um entsprechend ein erstes Steuersignal zur Positionskorrektur und/oder ein zweites Steuersignal zur Strömungsratenkorrektur zu erzeugen; und
eine entsprechende erste Korrektureinrichtung (14) zum Verschieben des Zentrums des Lichtstrahls zur Untersuchung zu dem Zentrum des Partikel-tragenden Fluids in der Strömungszellenvorrichtung in Übereinstimmung mit dem ersten Steuersignal und/oder eine zweite Korrekrureinrichtung (12) zum Korrigieren der Strömungsrate des Partikel-tragenden Fluids zu der vorbestimmten Strömungsrate in Übereinstimmung mit dem zweiten Steuersignal.

2. Fotoanalyseapparatur nach Anspruch 1, wobei die Markierer-Eingießeinrichtung (10) eine in der Strömung des Partikel-tragenden Fluids geöffnete Öffnung beinhaltet, und
die zweite optische Einrichtung aufweist:
einen Abtaster (15) mit einem Abtasterspiegel (2), der sich zwischen der Lampe und der Kondensorlinse befindet, zum Oszillieren des Lichtstrahls zur Untersuchung in einer kreuzenden Richtung der Strömung des Partikel-tragenden Fluids innerhalb eines vorbestimmten Winkels (α), wobei der Lichtstrahl zur Korrektur identisch ist mit dem oszillierten Lichtstrahl zur Untersuchung, einen ersten zwischen dem Abtasterspiegel und der Kondensorlinse angeordneten flachen Halbspiegel (3), der den Lichtstrahl zur Untersuchung durch sich hindurchtreten läßt und einen Lichtstrahl aus der entgegengesetzten Richtung des Lichtstrahls zur Untersuchung reflektiert, einen gekrümmten Halbspiegel (4), der zwischen dem ersten flachen Halbspiegel und der Kondensorlinse angeordnet ist, dessen konkave Oberfläche zu der Kondensorlinse zeigt, wobei der gekrümmte Halbspiegel einen Teil des Lichtstrahls zur Untersuchung durch sich hindurchtreten läßt und einen Teil des Lichtstrahls zur Untersuchung reflektiert, einen zweiten zwischen der Objektivlinse und dem ersten Fotodetektor angeordneten flachen Halbspiegel (8), der nur durch die Markierer in dem Partikel-tragenden Fluid verursachte Fluoreszenz oder Streulicht reflektiert, und einen zweiten Fotodetektor (9) zum Erfassen der durch den zweiten flachen Halbspieg l reflektierten Fluoreszenz oder Lichtstreuung, um das Signal zur Korrektur zu erzeugen, und
die erste Korrektureinrichtung eine Abtaster-Steuerungsvorrichtung (14) aufweist zum Steuern des Abtasters, um ein Zentrum der Oszillation in Übereinstimmung mit dem ersten Steuersignal zu ändern und
die zweite Korrektureinrichtung eine Ventil-Steuerungsvorrichtung (12) aufweist zum Steuern des Strömungs-Steuerventils, um die Strömungsrate des Partikel-tragenden Fluids in der Strömungszellenvorrichtung zu korrigieren in Übereinstimmung mit dem zweiten Steuersignal.

3. Fotoanalyseapparatur nach Anspruch 1, wobei die Markierer-Eingießeinrichtung zwei Öffnungen (28a, 28b) beinhaltet, die jeweils an jeder Seite der Strömung des Partikel-tragenden Fluids in der Strömungszellenvorrichtung geöffnet sind und von den beiden Öffnungen in das Hüllenfluid unterschiedliche Markierer eingießt, die Fluoreszenz oder Streulicht emittieren, deren Wellenlängen voneinander unterschiedlich sind, und
die zweite optische Einrichtung aufweist: einen zwischen der Lampe und der Kondensorlinse angeordneten Spiegel (2), wobei der Spiegel rotierbar ist, um den Lichtstrahl zur Untersuchung in einer kreuzweisen Richtung zu der Strömung des Partikel-tragenden Fluids zu bewegen, ein zwischen dem Spiegel und der Strömungszellenvorrichtung angeordnetes Beugungsgitter (26) zum Trennen von zwei Lichtstrahlen (29, 30) zur Korrektur von diesem Lichtstrahl zur Untersuchung, wobei die beiden Lichtstrahlen zur Korrektur jeweils an die Strömung (31, 32) der Markierer so angelegt werden, daß die jeweiligen Zentren der beiden Lichtstrahlen zur Korrektur von den jeweiligen Zentren der Strömung der Markierer in einer zueinander entgegengesetzten Richtung versetzt sind, einen zwischen der Objektivlinse und dem ersten Fotodetektor angeordneten flachen Halbspiegel (8), wobei der flache Halbspiegel nur durch die Markierer in dem Hüllenfluid verursachte Fluoreszenz oder Lichtstreuung reflektiert, und einen zweiten Fotodetektor (9) zum Erfassen von durch den ersten flachen Halbspiegel reflektierter Fluoreszenz oder Streulicht, um das Signal zur Korrektur zu erzeugen, und
die erste Korrektureinrichtung eine Steuerungsvorrichtung (27) aufweist zum Bewegen des Spiegels in Übereinstimmung mit dem ersten Steuersignal, und die zweite Korrektureinrichtung eine Ventil-Steuerungsvorrichtung(12) aufweist zum St uern des Strömungs-Steuerventils, um die Strömungsrate des Partikel-tragen-

den Fluids in der Strömungszellenvorrichtung in Übereinstimmung mit dem zweiten Steuersignal zu korrigieren.

4. Verfahren zum Korrigieren einer Positionsdifferenz zwischen einem Strömungzentrum von Partikel-(24)-tragendem Fluid (22) und einem Zentrum eines Lichtstrahls (18) zur Untersuchung und/oder zur Korrektur einer Strömungsrate des Fluids (22) in einer Fotoanalyseapparatur, welche aufweist: eine Strömungzelleneinrichtung (6) zum Eingießen von Hüllenfluid (23) und dem Partikel-tragenden Fluid in laminarer Strömung, in der das Partikeltragende Fluid durch das Hüllenfiuid umgeben ist, eine optische Einrichtung (1, 5, 7, 16) zum Anlegen des Lichtstrahls zur Untersuchung mit einer konstanten Wellenlänge an das Partikeltragende Fluid in der Strömungszelleneinrichtung, um die Stärke von durch die Partikel in dem Partikeltragenden Fluid verursachte Lichtstreuung und/oder Fluoreszenz zu messen, um ein Signal zur Untersuchung zu erzeugen, und eine Signalverarbeitungseinrichtung (17) zum Verarbeiten des Signals zur Untersuchung von der optischen Einrichtung, um Eigenschaften der Partikel, wie zum Beispiel Art, Größe, Anzahl und Form zu erfassen,
gekennzeichnet durch die folgenden Schritte:
Eingießen von Markierern, welche Fluoreszenz und/oder Streulicht mit einer konstanten Wellenlänge emittieren, wenn die Markierer einen Lichtstrahl (18; 29, 30) in dem Hüllenfluid (23) oder Partikeltragenden Fluid (22) empfangen,
Anlegen des Lichtstrahls (18; 29, 30) an die Markierer in der Strömungzelleneinrichtung, um die Stärke der durch die Markierer verursachten Fluoreszenz und/oder Lichtstreuung zu messen, um ein Signal zur Korrektur zu erzeugen;
Berechnen einer Positionsdifferenz von dem Signal zur Korrektur zwischen einem Zentrum des Partikel-tragenden Fluids und der Strömungzelleneinrichtung und einem Zentrum des Lichtstrahls zur Untersuchung und/oder einer Differenz der Strömungsrate zwischen einer vorbestimmten Strömungsrate und einer tatsächlichen Strömungsrate des Partikel-tragenden Fluids in der Strömungszelleneinrichtung, um entsprechend ein erstes Steuersignal zur Positionskorrektur und/oder eine zweites Steuersignal zur Strömungsratenkorrektur zu erzeugen; und
entsprechendes Verschieben des Zentrums des Lichtstrahls zur Untersuchung zu dem Zentrum d s Partikel-tragenden Fluids in der Strömungszelleneinrichtung in Übereinstimmung mit dem ersten Steuersignal und/oder Korrigieren der Strömungsrate des Partikel-tragenden Fluids zu der vorbestimmten Strömungsrate in Übereinstimmung mit dem zweiten Steuersignal.

5. Korrekturverfahren nach Anspruch 4, wobei
das Eingießen der Markierer ein Eingießen der Markierer in das Partikel-tragende Fluid (22) aufweist, und
das Anlegen des Lichtstrahls an die Markierer in der Strömungszelleneinrichtung ein Oszillieren des Lichtstrahls (18) zur Untersuchung in kreuzweiser Richtung zu der Strömung des Partikel-tragenden Fluids innerhalb eines vorbestimmten Winkels (α) aufweist.

6. Korrekturverfahren nach Anspruch 4, wobei
das Eingleßen der Markierer, das Eingleßen unterschiedlicher Markierer aufweist die Fluoreszenz und/oder Lichtstreuung emittieren mit konstanten voneinander unterschiedlichen Wellenlängen, wenn ein Lichtstrahl in dem Hüllenfluid (23) an jeder Seite der Strömung des Partikel-tragenden Fluids (22, 23) empfangen wird, und
das Anlegen des Lichtstrahls an die Markierer in der Strömungszelleneinrichtung, welches Trennen zweier Lichtstrahlen (29, 39) zur Korrektur von dem Lichtstrahl (18) zur Untersuchung aufweist, und zwar mittels eines Beugungsgitters (26), um sie jeweils an die Markierer an jeder Seite des Partikel-tragenden Fluids (22) anzulegen auf eine Art und Weise, daß Zentren der beiden Lichtstrahlen (29, 30) zur Korrektur von Zentren der Strömungen (31, 32) der Markierer in zueinander entgegengesetzten Richtungen versetzt sind.

Revendications

1. Appareil de photo-analyse comprenant:
des moyens de cellules d'écoulement pour déverser un fluide enveloppant (23) et un fluide (22) transportant des particules (24) dans un courant laminaire dans lequel ledit fluide (22) transportant des particules est enveloppé par ledit fluide enveloppant (23), ledit moyen de cellules d'écoulement comprenant un dispositif de cellules d'écoulement (6), et
des premiers moyens optiques (1, 5, 7, 16) pour appliquer un faisceau lumineux (18) d'examen ayant une longueur d'onde constant sur ledit fluide (22) transportant des particules dans le dispositif de cellules d'écoulement (6) afin de mesurer la force de dispersion de la lumière ou la fluorescence causée par lesdites particules (24) dans ledit fluide (22) transportant d s particules pour produire un

signal d'examen, lesdits premiers moyens optiques comprenant une lampe (1) pour émettre ledit faisceau lumineux (18) d'examen, une lentille de champ collectif (5) disposée entre ledit dispositif de cellules d'écoulement (6) et ladite lampe (1), une lentille d'objectif (7) disposée du côté opposé au dit dispositif de cellules d'écoulement (6), et un premier photo-détecteur (16) pour mesurer la force de dispersion de la lumière ou la fluorescence causée par lesdites particules (24) dans ledit fluide (22) transportant des particules pour produire un signal d'examen, et

des premiers moyens de traitement de signaux (17) pour traiter ledit signal d'examen desdits premiers moyens optiques pour détecter les caractéristiques des particules telle que sorte, dimension, nombre et forme:

caractérisé par

des moyens de déversement de marqueurs (10; 10a, 10b) pour déverser des marqueurs dans ledit fluide enveloppant (23) et le fluide (22) transportant des particules dans ledit dispositif de cellules d'écoulement (6), ledit marqueur émettant une fluorescence et/ou de la lumière dispersée ayant une longueur d'onde constante quand lesdits marqueurs reçoivent un faisceau lumineux (18; 29, 30) de correction;

une valve de contrôle de courant (13) pour contrôler la vitesse du fluide (22) transportant des particules;

des deuxièmes moyens optiques (2, 8, 9, 15; 26, 27) pour appliquer le faisceau de lumière de correction sur ledit marqueur dans le dispositif de cellules d'écoulement (6) pour mesurer la force de fluorescence et/ou de la lumière dispersée causées par ledit marqueur afin de produire un signal de correction;

des deuxièmes moyens de traitement de signaux (11) pour calculer à partir dudit signal de correction une différence de position entre un centre dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement et un centre dudit faisceau de lumière d'examen et/ou une différence dans la vitesse d'écoulement entre une vitesse d'écoulement prédéterminée et une vitesse d'écoulement réelle dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement afin de produire de façon correspondante un premier signal de contrôle pour la correction de la position et/ou un second signal de contrôle pour la correction de la vitesse d'écoulement; et

des premiers moyens de correction correspondants (14) pour déplacer le centre du faisc au lumineux d'examen vers le centre dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement en accord avec ledit premier signal de contrôle et/ou des seconds moyens de correction (12) pour corriger la vitesse d'écoulement dudit fluide transportant des particules vers une vitesse d'écoulement prédéterminée en accord avec ledit second signal de contrôle.

2. Appareil de photo-analyse selon la revendication 1, dans lequel lesdits moyens de déversement de marqueurs (10) contiennent une ouverture se trouvant dans l'écoulement dudit fluide transportant des particules, et

lesdits seconds moyens optiques comprennent un scanner (15) avec un miroir de scanner (2) qui est disposé entre ladite lampe et ladite lentille de champ collectif pour osciller ledit faisceau de lumière d'examen dans une direction transversale à celle de l'écoulement du fluide transportant des particules selon un angle (α) prédéterminé, ledit faisceau lumineux de correction étant identique audit faisceau lumineux d'examen oscillante, un premier demi-miroir (3) plat disposé entre ledit miroir de scanner et ladite lentille de champ collectif qui permet audit faisceau lumineux d'examen d'y passer à travers et reflète un faisceau lumineux de la direction opposée à celle dudit faisceau lumineux d'examen, un demi-miroir (4) courbé disposé entre ledit premier demi-miroir plat et ladite lentille de champ collectif, la surface concave de laquelle est tournée vers ladite lentille de champ collectif, le demi-miroir courbé permet à une partie dudit faisceau lumineux d'examen d'y passer à travers et reflète une partie dudit faisceau lumineux d'examen, un deuxième demi-miroir plat (8) disposé entre ladite lentille d'objectif et ledit premier photo-détecteur qui reflète seulement la fluorescence ou la lumière dispersée causée par ledit marqueur dans ledit fluide transportant des particules, et un deuxième photo-détecteur (9) pour détecter ladite fluorescence ou la lumière dispersée reflétée par ledit deuxième demi-miroir plat afin de produire ledit signal de correction, et

lesdits premiers moyens de correction comprennent un dispositif de contrôle (14) de scanner afin de contrôler ledit scanner pour changer le centre de l'oscillation en accord avec ledit premier signal de contrôle, et

lesdits deuxièmes moyens de correction comprennent un dispositif de contrôle de valve (12) afin de contrôler ladite valve de contrôle d'écoulement pour corriger la vitesse d'écoulement dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement

en accord avec ledit deuxième signal de contrôle.

3. Appareil de photo-analyse selon la revendication 1, dans lequel
lesdits moyens de déversement de marqueurs comprennent deux ouvertures (28a, 28b) respectivement disposées de chaque côté de l'écoulement du fluide transportant des particules dans ledit dispositif de cellules d'écoulement et déversent à partir de ces deux ouvertures dans le fluide enveloppant différents marqueurs qui émettent une fluorescence ou de la lumière dispersée ayant chacune une longueur d'onde différente, et
lesdits deuxièmes moyens optiques comprennent un miroir (2) disposé entre ladite lampe et la lentille de champ collectif, ledit miroir pouvant être tourné afin de bouger ledit faisceau lumineux d'examen dans une direction transversale à celle de l'écoulement du fluide transportant des particules, une grille de diffraction (26) disposée entre ledit miroir et ledit dispositif de cellules d'écoulement pour séparer deux faisceaux de lumière (29, 30) pour la correction dudit faisceau de lumière d'examen, lesdits deux faisceaux de lumière de correction étant respectivement appliqués sur le courant (31, 32) de marqueurs de façon à ce que les centres respectifs des deux faisceaux de lumière de correction soient déplacés par rapport à l'écoulement des marqueurs dans des directions opposées l'une à l'autre, un demi-miroir (8) plat disposé entre ladite lentille d'objectif et ledit premier photo-détecteur, ledit demi-miroir plat reflètant seulement la fluorescence ou la lumière dispersée causée par lesdits marqueurs dans ledit fluide de protection, et un deuxième photo-détecteur (9) pour détecter la fluorescence ou la lumière dispersée reflétée par ledit demi-miroir plat afin de produire un signal de correction, et
lesdits premiers moyens de correction comprennent un dispositif de contrôle (27) pour déplacer ledit miroir en accord avec ledit premier signal de contrôle, et lesdits deuxièmes moyens de correction comprennent un dispositif de contrôle (12) de valve afin de contrôler ladite valve de contrôle d'écoulement pour corriger la vitesse d'écoulement dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement en accord avec ledit deuxième signal de contrôle.

4. Procédé pour corriger une différence dans la position entre un centre d'écoulement d'un fluide (22) transportant des particules (24) t un centre d'un faisceau de lumière (18) d'examen et/ou pour corriger une vitesse d'écoulement du fluide (22) dans un appareil de photo-analyse qui comprend un dispositif de cellules d'écoulement (6) pour déverser un fluide enveloppant (23) et le fluide transportant des particules dans un courant laminaire dans lequel le fluide transportant des particules est enveloppé par le fluide enveloppant, des moyens optiques (1, 5, 7, 16) pour appliquer un faisceau de lumière d'examen ayant une longueur d'onde constante sur le fluide transportant des particules dans le dispositif de cellules d'écoulement pour mesurer la force de dispersion de la lumière et/ou de fluorescence causée par les particules dans le fluide transportant des particules afin de produire un signal d'examen, et des moyens de traitement de signaux (17) pour traiter le signal d'examen des moyens optiques pour détecter les caractéristiques des particules telles que sorte, dimension, nombre et forme,
caractérisé par les étapes suivantes
déverser des marqueurs qui émettent une fluorescence et/ou de la lumière dispersée ayant une longueur d'onde constante quand les marqueurs reçoivent un faisceau de lumière (18; 29, 30) dans ledit fluide de protection (23) ou dans le fluide (22) transportant des particules,
appliquer le faisceau de lumière (18; 29, 30) sur ledit marqueur dans ledit dispositif de cellules d'écoulement afin de mesurer la force de fluorescence et/ou de la lumière dispersée causée par lesdits marqueurs afin de produire un signal de correction;
calculer à partir dudit signal de correction une différence dans la position entre un centre dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement et un centre dudit faisceau de lumière d'examen et/ou une différence dans la vitesse d'écoulement entre une vitesse d'écoulement prédéterminée et une vitesse d'écoulement réelle dudit fluide transportant les particules dans ledit dispositif de cellules d'écoulement afin de produire de façon correspondante un premier signal de contrôle pour la correction de la position et/ou un deuxième signal de contrôle pour la correction de la vitesse d'écoulement; et
déplacer de façon correspondante le centre du faisceau lumineux d'examen vers le centre dudit fluide transportant des particules dans ledit dispositif de cellules d'écoulement en accord avec ledit premier signal de contrôle et/ou corriger la vitesse d'écoulement dudit fluide transportant des particules vers une vitesse d'écoulement prédéterminée en accord avec ledit deuxièm signal de contrôle.

5. Procédé de correction selon la revendication 4, dans lequel
ledit déversement de marqueurs comprend le déversement de marqueurs dans ledit fluide (22) transportant des particules et
ladite application du faisceau lumineux sur lesdits marqueurs dans ledit dispositif de cellules d'écoulement comprend l'étape d'osciller ledit faisceau lumineux (18) d'examen dans une direction transversale à celle de l'écoulement du fluide transportant des particules selon un angle (α) prédéterminé.

6. Procédé de correction selon la revendication 4, dans lequel
ledit déversement de marqueurs comprend le déversement de différents marqueurs qui émettent une fluorescence et/ou une lumière dispersée ayant une longueur d'onde constante différente l'une de l'autre quand un faisceau lumineux est reçu dans le fluide enveloppant (23) de chaque côté de l'écoulement du fluide (22) transportant des particules, et
ladite application du faisceau lumineux sur lesdits marqueurs dans ledit dispositif de cellules d'écoulement comprend la séparation de deux faisceaux lumineux (29, 30) de correction dudit faisceau lumineux (18) d'examen au moyen d'une grille de diffraction (26) pour les appliquer respectivement sur lesdits marqueurs de chaque côté du fluide (22) transportant des particules de façon à ce que les centres des deux faisceaux lumineux (29, 30) de correction soient déplacés par rapport aux centres des courants (31, 32) des marqueurs dans des directions opposées l'une à l'autre.

FIG. 1

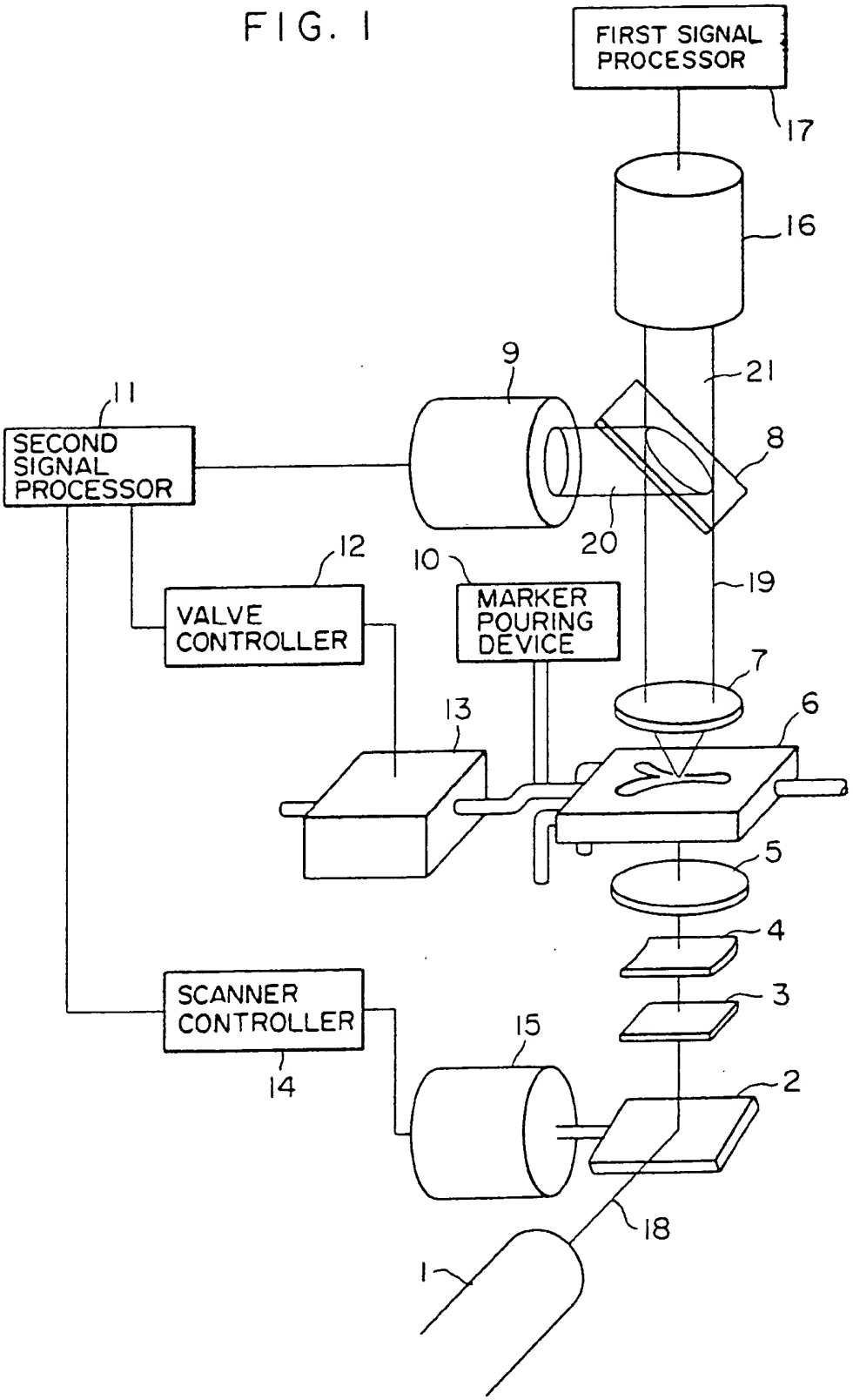

FIRST SIGNAL PROCESSOR
17
16
21
9
11
SECOND SIGNAL PROCESSOR
8
20
12
10
VALVE CONTROLLER
MARKER POURING DEVICE
19
7
13
6
5
4
3
SCANNER CONTROLLER
15
14
2
18
1

FIG. 2

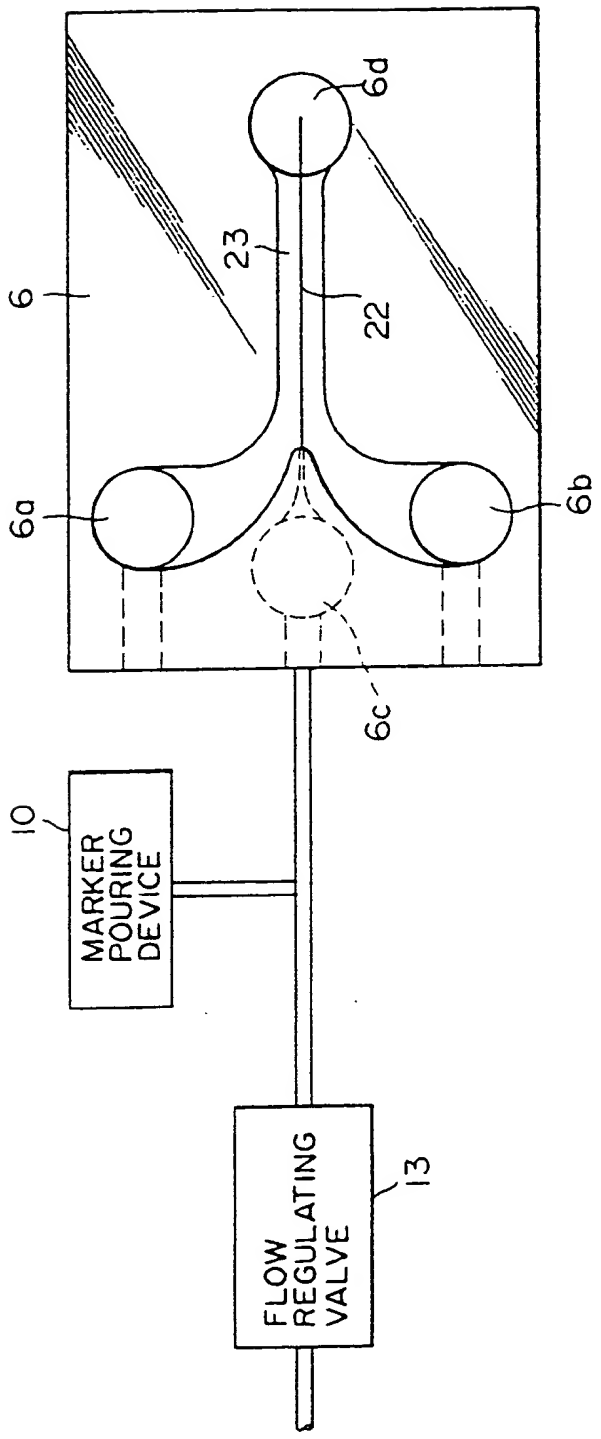

6d
23
22
6
6a
6b
6c
10
MARKER POURING DEVICE
FLOW REGULATING VALVE
13

FIG. 3

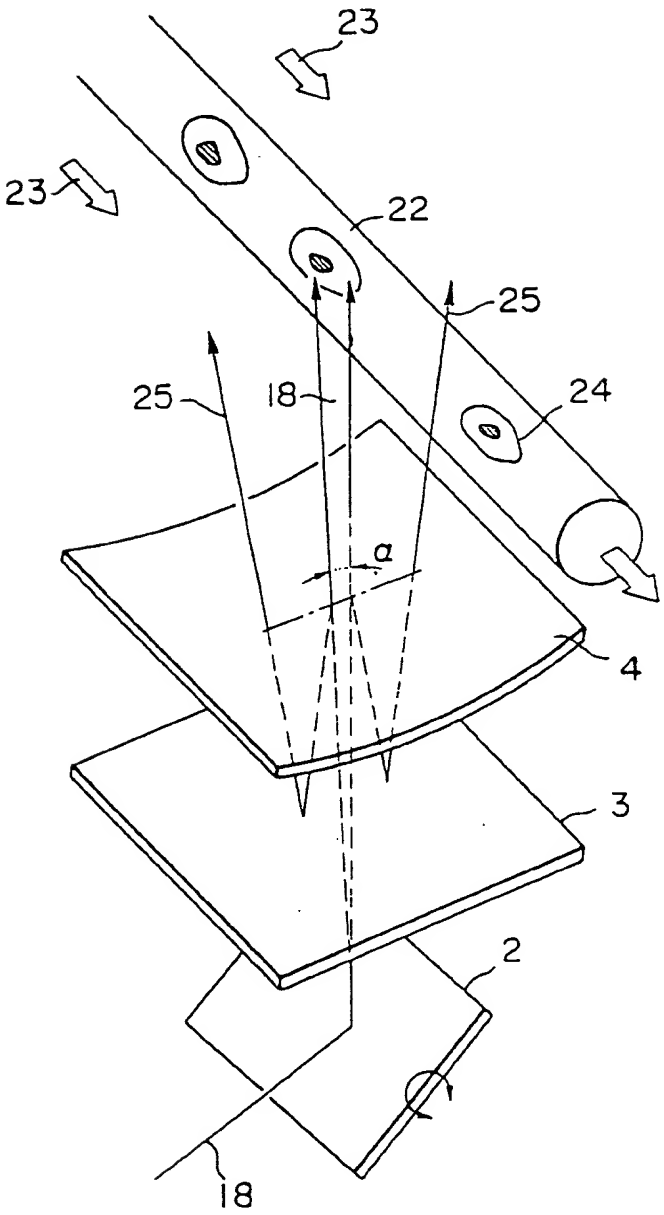


FIG. 4-1-a

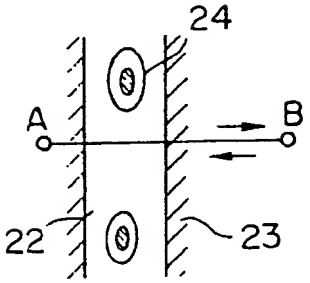


FIG. 4-1-b

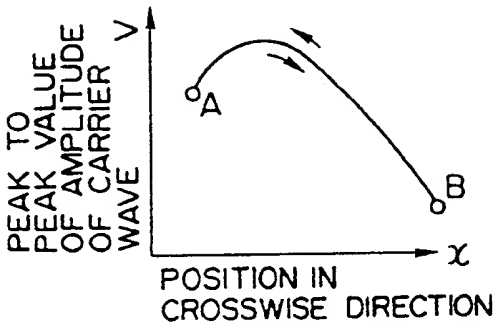


FIG. 4-1-c

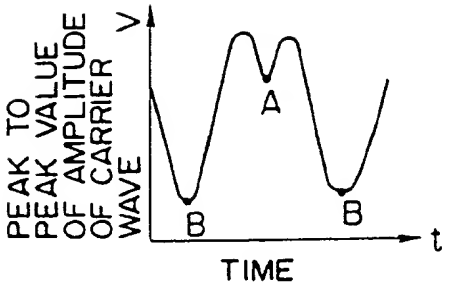


FIG. 4-1-d

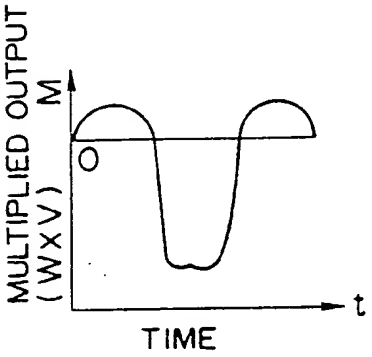


FIG. 4-1-e

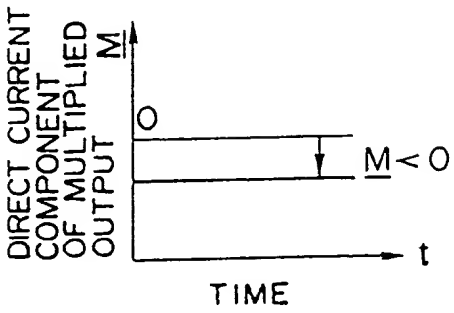


FIG. 4-2-a

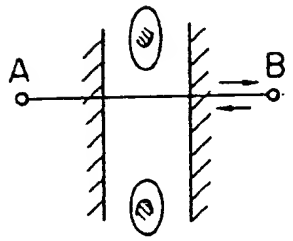


FIG. 4-2-b

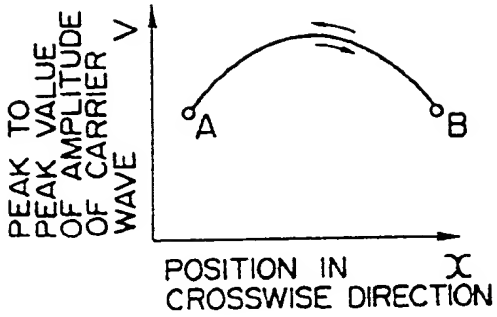


FIG. 4-2-c

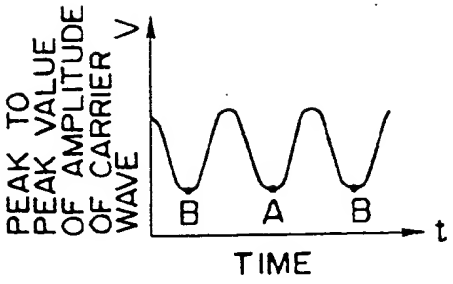


FIG. 4-2-d

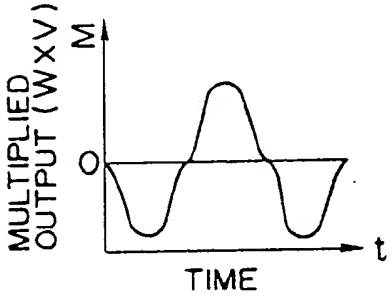


FIG. 4-2-e

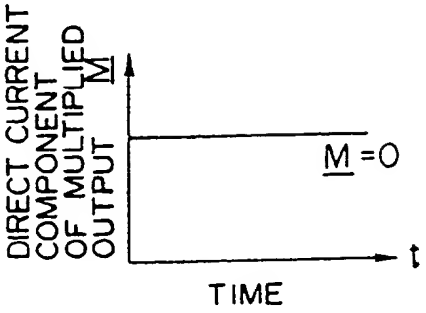


FIG.4-3-a

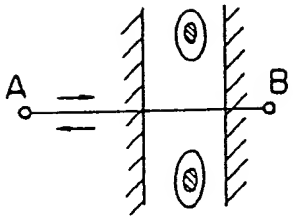


FIG.4-3-b

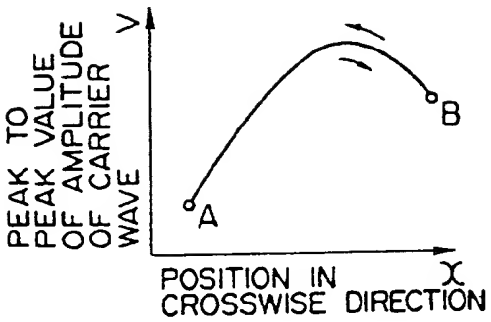


FIG.4-3-C

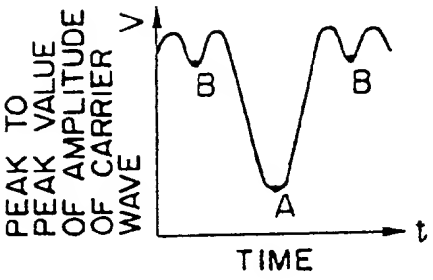


FIG. 4-3-d

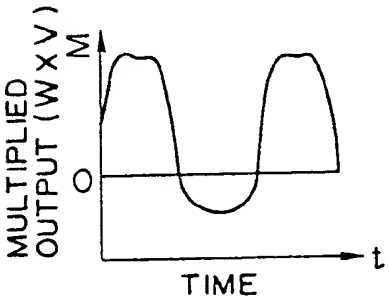


FIG.4-3-e

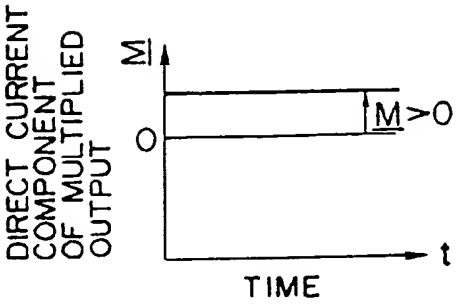


FIG. 4-4

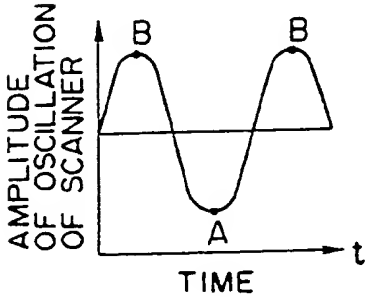


FIG. 5-I-a

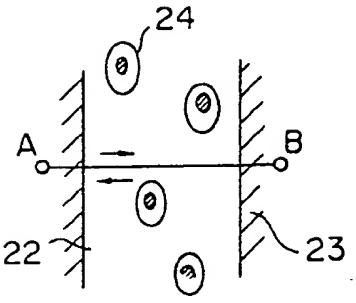


FIG. 5-I-b

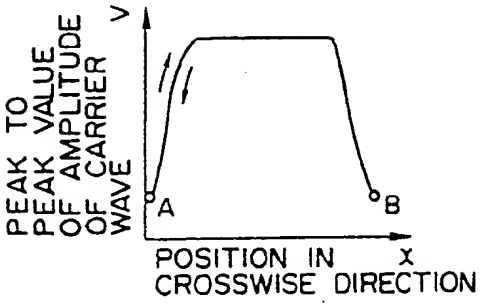


FIG. 5-I-C

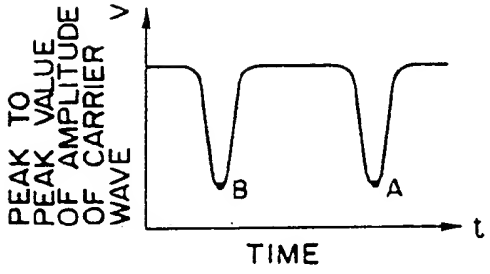


FIG. 5-I-d

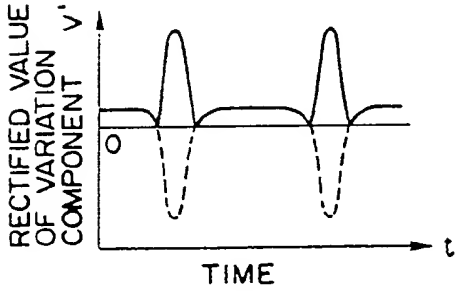


FIG. 5-I-e

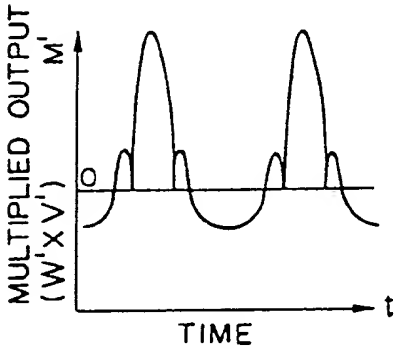


FIG. 5-I-f

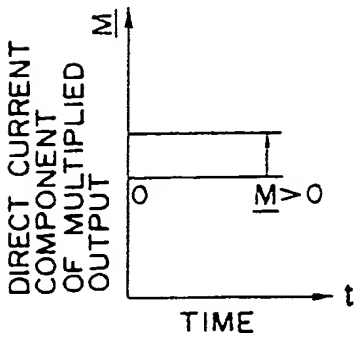


FIG.5-2-a

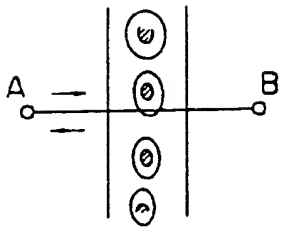


FIG.5-2-b

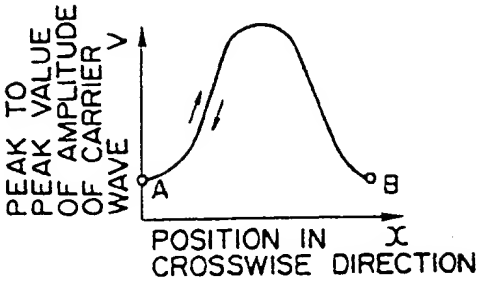


FIG.5-2-c

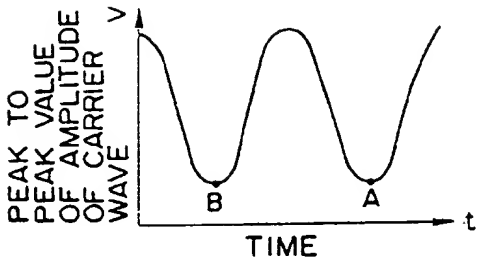


FIG.5-2-d

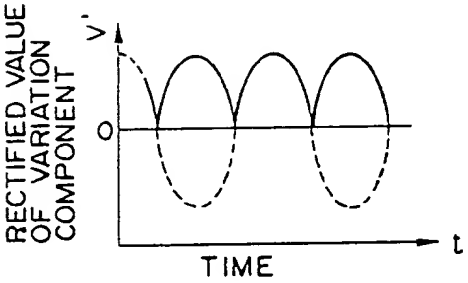


FIG.5-2-e

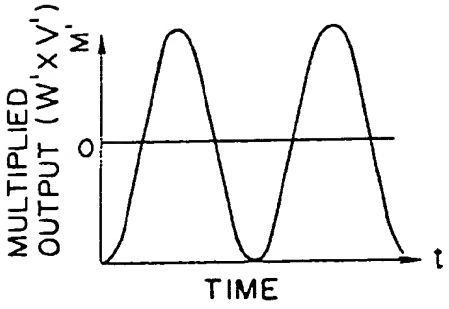


FIG.5-2-f

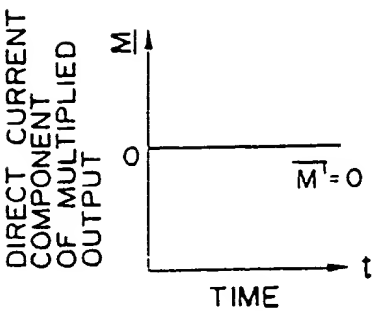


FIG. 5-3-a

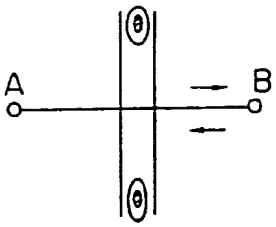


FIG. 5-3-b

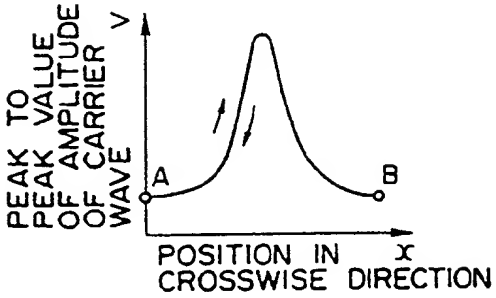


FIG. 5-3-c

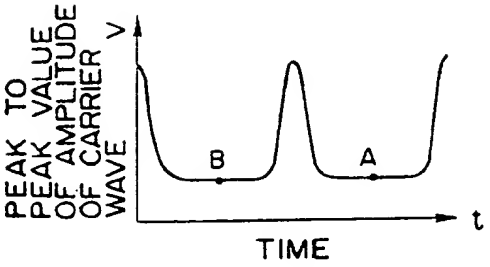


FIG. 5-3-d

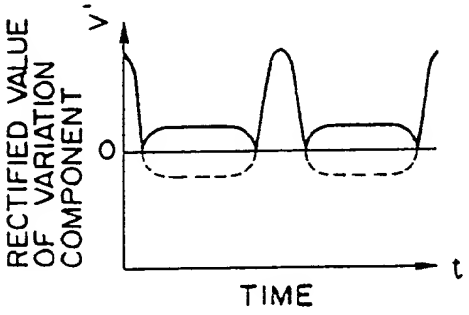


FIG. 5-3-e

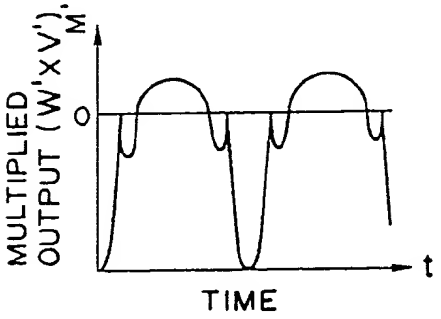


FIG. 5-3-f

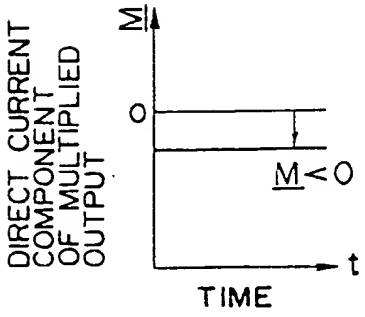


FIG. 5-4

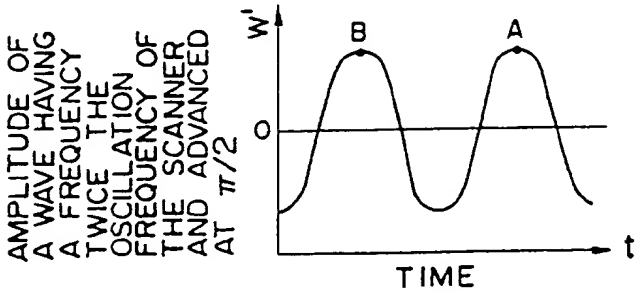
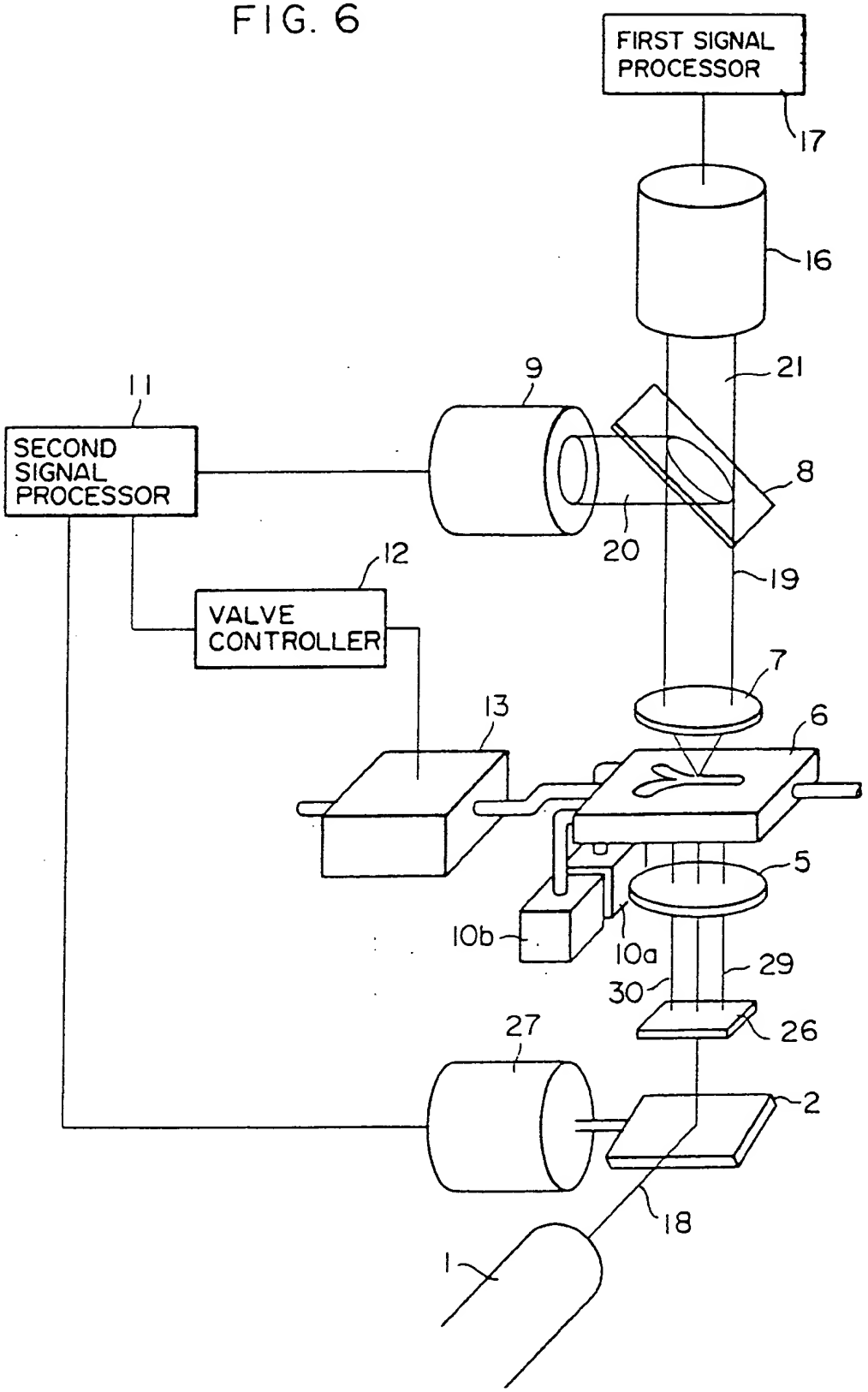


FIG. 6


FIRST SIGNAL PROCESSOR
17
16
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SECOND SIGNAL PROCESSOR
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VALVE CONTROLLER
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10b
10a
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FIG. 7

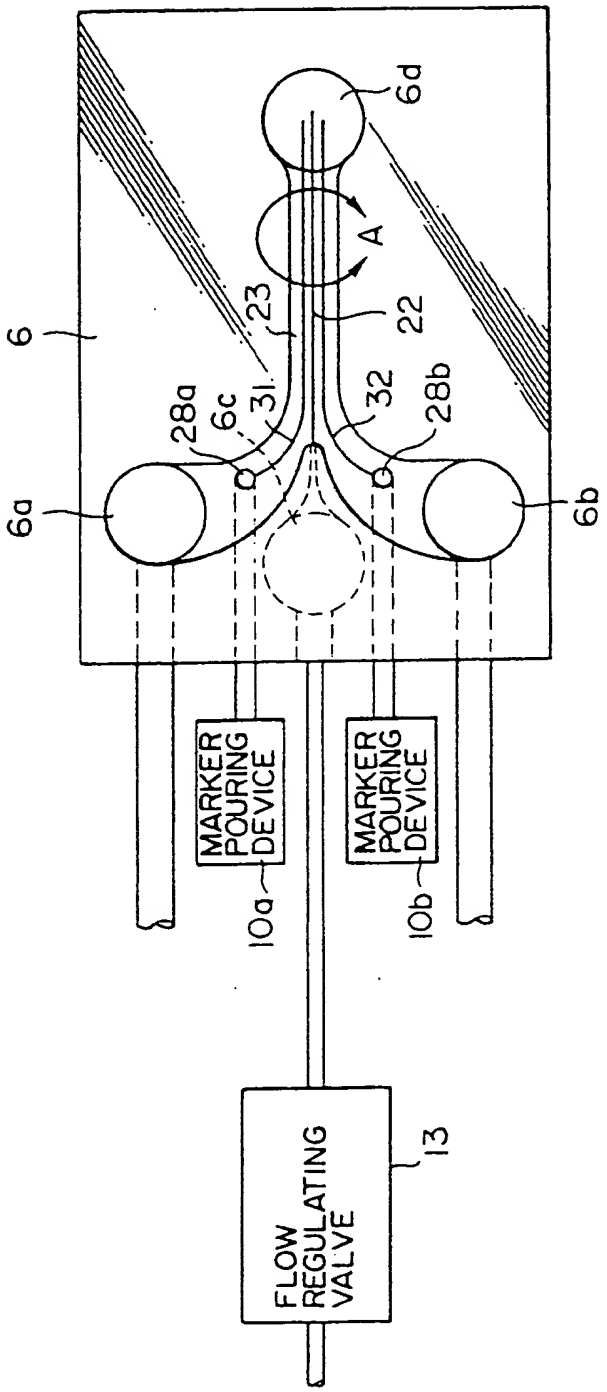
6
6a
6b
6d
A
22
23
31
32
28a
28b
6c
MARKER POURING DEVICE
MARKER POURING DEVICE
10a
10b
FLOW REGULATING VALVE
13

FIG. 8 a

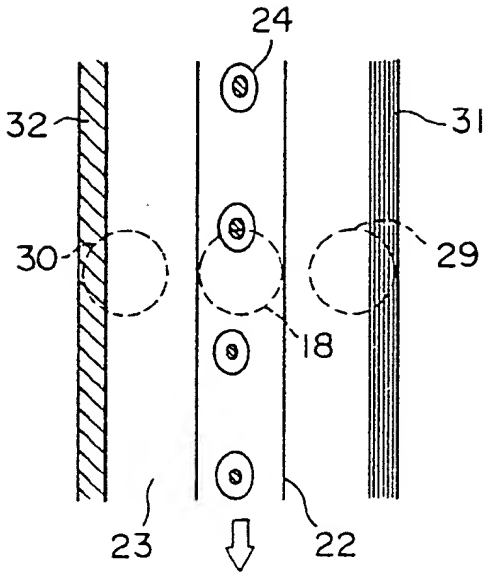

FIG. 8 b